MEETING REPORT

MOLECULAR GENETICS OF ANTIBODY FORMATION

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Immunology has been an art since time immemorial, and a science for approximately one hundred years. The recent development of immunology has been tremendous, even when compared with other areas of biology. Much of it has been on a molecular, i.e. a chemical, level and most recently many cellular immunological phenomena became prone to a molecular attack. Out of the manifold *hot* spots of immunology the organizers of the 7th Meeting of the Federation of European Biochemical Societies in Varna (Bulgaria) last September chose the Molecular Genetics of Antibody Formation [1] as the topic for an immunochemical colloquium (organized by M. Sela, Rehovot and M. Yomtov, Sofia). The first part of the colloquium dealt with the problems of the structural genes for immunoglobulins, the generation of their diversity and the chemical and serological differences between various immunoglobulins, including known antibodies. The second part of the colloquium was devoted to the determinant–specific genetic control of immune responsiveness.

The efforts to understand the biological expression of immunological phenomena through investigations at a precise chemical level makes it particularly appropriate to recall the visionary words of the great Swedish physical chemist, Svante Arrhenius, in his book "Immunochemistry" published in 1907 [2]. It was Arrhenius who first coined the expression *immunochemistry*. His interest in the immunological field was triggered by Paul Ehrlich's investigations of the reactions of toxins with antitoxins. Arrhenius saw that they obeyed the law of mass action and initiated a comprehensive study of the problem. But the immunobiologists of that period, including Ehrlich himself, became critical of his approach. To this, Arrhenius answers in the preface of his book [2]:

"It is evident that the objection recently raised by Ehrlich and Sachs to this manner of investigation, namely, that it does not enter upon the mode by which the living body produces these so-called antibodies, is quite true. An investigation of the chemical relations of toxin and antitoxin need not carry with it an elucidation of the synthesis of the antitoxin. But I fancy that there are many who are so deeply interested in the chemical behaviour of these substances that they will find an investigation of this question well worthy of study. And for myself, furthermore, I believe that the physiological side of the problem, alluded to by Ehrlich, will not find a satisfactory solution until the more simple chemical aspect is elucidated."

N. Hilschmann (Göttingen) presented an impressive barrage of monoclonal immunoglobulin sequence data from his own laboratory and correlated them with information available from other laboratories, presenting a convincing case for the germ line theory of antibody formation. There was, nevertheless, no unanimity of opinion, and F. Franěk (Prague) in his concluding remarks jokingly suggested that "the safest theories are partly germ-line and partly somatic,

as their authors will always be able to say that they were correct – partly". Whatever the final validity of the various theories, there is no doubt that, due to its division into a constant and a variable part, *v*, the genetic control of the immunoglobulin molecule is more complicated than with other proteins. It is unique in this respect that one immunoglobulin peptide chain is controlled by two genes: one for the constant and one for the variable part. On the basis of a discriminating chemical homology subgroups and "subsubgroups" can be recognized within the variable parts. Proteins belonging to one subgroup have many more residues in common than proteins belonging to different subgroups (and this is true also for subsubgroups). Hilschmann's argument is that this regularity must be caused by evolution and not by a somatic hypermutation process, since independent mutations in different individuals would not have occurred in a parallel way. The only somatic step Hilschmann admits during cell differentiation is the fusion of one of the variable genes with the corresponding gene for the constant part. This process is essential in cell differentiation, forming a unipotent antibody synthesizing cell from a multipotent stem cell.

The structural basis of allotype specificity in rabbit immunoglobulin G was discussed by R.R. Porter (Oxford), whose laboratory has investigated in detail the A1, A2, A3 locus. Some 12–14 positions in the variable region of the heavy chain show a correlation between the amino acid residue present and the allotype of the protein. This is true for pooled and for individual immunoglobulin preparations. The origin of these differences, necessitating two base changes of the codon in several cases, implies that rapid mutation was occurring when they arose and yet now they show complete stability. At present this seems inexplicable, but, in contrast to the conclusions of Hilschmann, Porter thinks that it is impossible to reconcile the presence of 14 stable amino acid positions in the variable region with the germ line theory.

A serological and chemical study of the same group *a* allotypic specificities in rabbits was described by J.W. Prahl (Pasadena) and C.W. Todd (Los Angeles). Whereas normally 80–90% of the heavy chains of the IgG of a rabbit carry these specificities, they have succeeded in suppressing them in rabbits homozygous at the group *a* locus by embryo transfer to a surrogate mother lacking their allotypic genome, followed by injecting the resulting neonates with anti-allotype serum. The immunoglobulins of rabbits suppressed in this manner lack group *a* specificities, and Prahl calls them "blank" immunoglobulins. It is interesting to speculate about the structural reasons for the lack of cross-reaction of the new immunoglobulin with known anti-allotype sera. Are there many new amino acid replacements in the positions characteristic of this allotype? Or is it a change in the amino acid sequence which causes a transformation of the molecule and thus does not allow the reaction? The suppressed heavy chain was found to possess a different amino-terminal sequence, namely, pyrrolidone carboxylic–glutamyl–glutamine. It would be desirable to obtain antibodies reacting specifically only with the "blank" immunoglobulin. Should all rabbits possess a small amount of the blank IgG, this might become worth trying, possibly by immunization of other species, followed by appropriate immunoadsorptions.

In contrast to the rabbit, genetic markers of *human* immunoglobulin chains have been found in this constant region, and M.W. Turner (London) reported on his and J.B. Natvig's (Oslo) successful efforts to map the markers more precisely by using pepsin-produced segments. The role of steric conformation in preserving the genetically relevant antigenic determinants is apparent from the loss of reaction upon further proteolytic digestion.

Most interesting was the talk by J. Oudin (Paris), the discoverer of rabbit allotypes and idiotypes. Idiotypy of antibodies is their property of possessing antigenic specificities which differ according to the antigens against which they are directed and according to individuals, or perhaps groups of individuals, in which they are produced. The antigens used by Oudin were *Salmonella typhi, Salmonella abortus-equi* and ovalbumin. The same idiotypic pattern has been observed in antibodies of a unique specificity which belonged to the IgG and IgM classes. Idiotypy may change with the course of immunization as was shown by, for example, that two idiotypic patterns which are carried by two distinct molecules may be carried in serum from a previous bleeding by a single molecule. A particularly fascinating novel observation from Oudin's laboratory is that the same idiotypic specificity may be

found on antibodies differing in their affinity and possibly even in their specificity. This conclusion was arrived at from careful studies of anti-ovalbumin antibodies, including selective immunoadsorption and elution according to different antigenic determinants (using duck, turkey and hen ovalbumin) and different affinities (using different molarities of magnesium chloride for elution). Are the immunoglobulins possessing the same idiotype, but apparently devoid of any anti-ovalbumin reactivity, antibodies with some other antigenic specificity, or are they failed anti-ovalbumins?

A.R. Williamson (London) described the use of antibody isoelectric spectra as phenotypic markers for the variable regions of anti-hapten antibodies. The question asked was: how many different anti-hapten (NIP) molecules can an inbred strain of mice (CBA) make? A cell transfer system was used to distribute the secondary response among many recipients such that each had about two clones, on the average. A large number of unique antibodies were seen with a low incidence ($< 2\%$) of repetition of the same antibody from different donors. At this stage Williamson concludes that the total possible number of anti-NIP molecules should be between 10^4 and 10^5. This large number could, however, be generated by only 200 variable regions each for light and heavy chains. Such a number of variable genes could easily be carried in the germ-line.

The immune state of an individual is not in itself an inherited characteristic. Nevertheless, the ability of an animal to elicit an immune response to a specific immunogen is subject to genetically determined factors. Results of investigations reported during the last decade have indicated that the immune response potentials of several rodent species to natural and synthetic immunogens are under genetic regulation [3]. While the whole subject of the genetic control of immune response has been under vigorous investigation only in the last few years, it is pertinent to recall that the topic was mentioned in the literature as early as 1916, when Cooke and Vander Veer, Jr., published a paper entitled "Human Sensitization" [4]. I quote: "It has been found that in cases of bilateral inheritance a larger proportion of the children become sensitized at an average earlier age than in cases of unilateral notwithstanding the fact that in more than one-third of the former group the hereditary influence on one side of the family was not seen in the parent, but in a grandparent or a collateral, such as an uncle. It is apparent here that the parent not clinically affected has transmitted some characteristic to his offspring the nature of which cannot be specified. It is however conceivable that such parents have *latent* sensitization." The authors then ask themselves whether their cases "might conform to the Mendelian laws either as a dominant or as a recessive characteristic" and conclude that their data strongly "suggest that sensitization is inherited as a dominant characteristic."

The first example in which unigenic control of immune response has been demonstrated is the "PLL gene" in guinea pigs [3]. In this system, the genetic regulation of the immune response to poly-L-lysine and to a random copolymer of L-glutamic acid and L-lysine, as well as hapten conjugates of these polymers, was found to be determined by a single autosomal gene. A dominant, quantitative, determinant-specific genetic control of antibody response has been observed in inbred mouse strains using branched chain synthetic polypeptide immunogens in which short peptides of glutamic acid and tyrosine, histidine or phenylalanine were attached to DL-alanine side chains, which were linked to a poly-L-lysine backbone. Mice of the C57BL strain were found to be high responders to the tyrosine-containing polypeptide, abbreviated as (T,G)-A--L, whereas they were low responders to the macromolecule containing histidine, (H,G)-A--L. Conversely, CBA mice were high responders to (H,G)-A--L, but low responders to (T,G)-A--L. The (C57 X CBA) F_1 hybrids responded well to both immunogens, and the backcross progeny segregated in response to (T,G)-A--L and (H,G)-A--L as a 1:1 mixture of the F_1 and respective homozygous parents.

Expression of genetic control of immune responsiveness has been demonstrated at the cellular level, since irradiated low responder mice and guinea pigs generated responses characteristic of the high responder strains after injection of lymphoid cells from high responder donors. The response potentials of guinea pigs for poly-L-lysine and of mice for the A--L series of immunogens are both closely linked to the respective major histocompatibility regions of these two species. The responses of inbred mice

were different and not linked to H-2 when the poly-DL-alanine side chains were replaced with poly-L-proline, even though the same short peptide sequences were attached to polyproline. Genetic control of antibody specificity has been demonstrated using the synthetic polypeptide poly-(Phe, Glu)-poly-Pro--poly-Lys, denoted (phe, G)-Pro--L. Two different mouse strains responded well to (Phe, G)-Pro--L, but the antibodies elicited were of different specificities. DBA/1 mice responded mainly to the (Phe,G) part of the immunogen, whereas most of the antibodies produced in the SJL strain were specific for Pro--L.

M. Sela (Rehovot) described studies, with E. Mozes and G.M. Shearer, in which it was established that the low immune response to the (Phe,Glu) and the polyproline regions of (Phe, G)-Pro--L could be correlated with a reduced number of detectable splenic antigen-sensitive precursors. The reduction in frequency could be attributed to the population of cells derived from bone marrow, and not from thymus. On the other hand, using the same techniques involving transfer into irradiated recipients of graded inocula of cells of one type from syngeneic donors while giving an excess of cells of the other type (thymus versus bone marrow), it could be shown that in the case of (T, G)-A--L the genetic defect is expressed in cells derived both from thymus and bone marrow.

Immune response to antigens based on polyproline could be corrected in the poor responder strains of mice with the double-stranded polyadenylic–polyuridylic acid, as well as with peritoneal exudate cells. Neither of these two was able to influence the poor response to antigens based on poly-DL-alanine. From these, and other observations, it was concluded that the mechanism of the genetic control of immune response depends heavily on the chemical structure of the immunogen.

I. Říha (Prague) reported on dominant genetically controlled differences in antibody formation in mice towards two haptens: *p*-aminobenzoic acid and sulfanilic acid. The differences in anti-hapten response were not due to differences in the immunogenicity of the protein-carrier in individual strains. In agreement with Sela's report on the genetic control of antibody response to polyproline-derived antigens (but not polyalanine-derived) Říha finds that the bone marrow cells are mainly responsible for the high or low response to the hapten. Říha believes that the genetic defect is on the level of presence or absence of some structural genes, leading in the poor responders to antibodies of lower affinity and/or more restricted heterogeneity. It seems to me that the distinct possibility that the same structural genes are present both in low and high responder strains, and that the genetic control of the immune response occurs at some step other than the structural gene, cannot at this stage be discarded, and should be actively investigated.

Another interesting report was that of E. Kölsch (Hamburg) on the genetics of immune response to bacteriophage *fd* in mice. The genetic defect is determinant-specific, and not linked to H-2 specificity locus. The heterogeneity of antibodies produced in the low responder strains is more restricted than in the high responders. Polyacrylic acid restores the immune response to *fd* in some strains, but not in others.

Genetically predetermined differences in antibody formation in inbred strains of mice in response to immunization with non-pathogenic leptospirae and with sheep red blood cells were observed by R.V. Petrov (Moscow) and his colleagues. Even though Dr. Petrov did not attend the meeting he made a copy of his presentation available to the organizers. For both antigens the high response capacity was inherited as a dominant trait controlled by more than one gene. From experiments with cell transfers into lethally irradiated mice Petrov concludes that certain cells of recipients are radio-resistant and that their presence is crucial in immune response. His working hypothesis is that this cell is the macrophage.

It may be said in conclusion that we are still largely in the dark concerning the nature and mechanism of the genetic control of immune responsiveness, even though a lot of important information has been accumulated very recently. Let us hope that before the next FEBS Meeting convenes a clue will be found that will permit at least more informed guesses concerning this fascinating phenomenon.

References

[1] Abstr. Commun. 7th Meet. Fed. Eur. Biochem. Soc., published by the Bulgarian Biochemical and Biophysical Society (1971) p. 79.

[2] Svante Arrhenius, Immunochemistry (Macmillan Co., New York, 1907).
[3] H.O. McDevitt and B. Benacerraf, Adv. Immunol. 11 (1969) 31.
[4] R.A. Cooke and A. Vander Veer, Jr., J. Immunol. 1 (1916) 201.